

AN EMBRYOLOGICAL STUDY OF A
SPECIAL STRAIN OF DEFORMED
X-RAYED MICE, WITH SPECIAL
REFERENCE TO THE ETIOLOGY
AND MORPHOGENESIS OF
THE ABNORMALITIES

BY
GEORGE M. PLAGENS

A dissertation submitted in partial fulfillment of the requirements for the
degree of Doctor of Science in the University of Michigan

Reprinted from the JOURNAL OF MORPHOLOGY
Vol. 55, No. 1, September, 1933

AN EMBRYOLOGICAL STUDY OF A
SPECIAL STRAIN OF DEFORMED
X-RAYED MICE, WITH SPECIAL
REFERENCE TO THE ETIOLOGY
AND MORPHOGENESIS OF
THE ABNORMALITIES

BY
GEORGE M. PLAGENS

A dissertation submitted in partial fulfillment of the requirements for the
degree of Doctor of Science in the University of Michigan

Reprinted from the JOURNAL OF MORPHOLOGY
Vol. 55, No. 1, September, 1933

*29454



AN EMBRYOLOGICAL STUDY OF A SPECIAL STRAIN
OF DEFORMED X-RAYED MICE, WITH SPECIAL
REFERENCE TO THE ETIOLOGY AND
MORPHOGENESIS OF THE
ABNORMALITIES ¹

GEORGE M. PLAGENS

FOUR PLATES (THIRTEEN FIGURES)

AUTHOR'S ABSTRACT

This investigation consists of an extensive histological study of a special strain of abnormal x-rayed mice, in an attempt to determine the embryonic origin and development of certain congenital abnormalities that are hereditary.

The study shows that development of the x-rayed strain is normal up to the thirteenth day after insemination, after which pathological structures appear in the form of blebs, hematomas, and thrombi. As a result of the formation of the blebs and hematomas a general condition is set up within the embryo which resembles that of thrombosis. The thrombi when formed exert a mechanical effect by crowding out the normal tissue. A chemical effect is also produced by the blood cells and fluid extravasated from the thrombus which penetrate into the forming tissues resulting in their perverted development. Thus the thrombi are the immediate factors causing the various regions of the body to be deformed.

INTRODUCTION

Formerly malformations were recognized only as 'freaks of nature,' and were regarded as of no importance other than as scientific curiosities, but today these conditions challenge the embryologist's interest, since they throw a new light on the factors governing normal differentiation and growth.

Congenital maldevelopments are the result of arrests or aberrances in intra-uterine development, but the factors involved in inhibiting or preventing the normal embryonic events are not wholly understood. The cause may be evident in certain instances, but in a large percentage of cases, at the present time, the etiology is obscure. The congenital malformation is of prenatal or parturitive origin and is due either to some intrinsic factor (hereditary or otherwise) in

¹ Contribution from the Zoölogical Laboratory of the University of Michigan.

the fetus, or to some extrinsic factor in the uterine environment. In any event, the anomalies probably are the result of localized nutritional disturbances, or defective conditions of a chemical, physical, or bacteriological nature. A great many cases, no doubt, may be attributed to specific diseases, but to this must be added the tendency to inherit cellular differences which predispose individuals not only to diseases, but to developmental anomalies as well.

If congenital anomalies are inherited, and there is good reason to believe that heredity plays a more conspicuous role etiologically than any other factor, then the best opportunity of identifying the morphological factors underlying this condition lies in an intensive study of the 'histories' of the families in which such anomalies appear. And it would be of particular advantage to know the nature of the developmental processes present at different periods of gestation in fetuses predestined by heredity to show some type of anomaly. The information hitherto obtained relating to this subject has been exceedingly fortuitous, as such hereditary abnormalities have been observed and studied usually only in man, where sufficient historical data are often unobtainable.

It has long been hoped that some one would find hereditary abnormalities in some strain of lower mammals, and that the defect and the tendency toward its recurrence would be so pronounced that one could investigate many phases of the problem which are open to investigation only in species where large numbers of individuals are available. Such material has been found in a strain of mice derived from an x-rayed stock.

The purpose of this paper is to describe the embryonic origin and development of certain defects occurring as congenital abnormalities in the descendants of a special strain of x-rayed mice. Some phases of this problem have received special consideration by Little and Bagg ('23, '24), Bagg ('29), and Bonnevie ('31). The pertinent literature will be considered in connection with the presentation of the results of the present investigation.

ACKNOWLEDGMENTS

Throughout the course of the investigation here recorded I have been fortunate in having the constant assistance and direction of Dr. Peter Okkelberg, whose suggestions have been of utmost value. For the initial impetus to the work I am indebted to Dr. C. C. Little. His courtesy in providing the stock of mice and teaching me the technique of breeding and caring for them is hereby acknowledged.

I desire also to express my deepest gratitude and appreciation to Dr. Carl V. Weller, director of the Pathological Laboratory, University of Michigan, and to Dr. Raphael Isaacs, assistant director of the Simpson Memorial Institute, University of Michigan, for their invaluable assistance in the interpretation of the pathological conditions recognized in the material. I am also greatly indebted to Dr. A. Franklin Shull, professor of zoölogy, University of Michigan, for his assistance in the interpretation of the statistical data.

HISTORY

Many hypotheses have been formulated concerning the etiology of congenital abnormalities, but no one has yet succeeded in advancing a theory that encompasses all cases, agrees with the facts of embryology, and explains satisfactorily the pathological conditions involved.

Martin (1839) and Velpeau (1847) attributed congenital deformities to a deficiency of the amniotic fluid, as a consequence of which the uterus contracts forcibly upon the developing fetus causing various parts to become deformed. This view was questioned by Adams (1873) on the grounds that in many cases of deficient amniotic fluid perfectly formed infants are born. Congenital deformities have been referred to possible malpositions or abnormal pressures in utero of such long duration as to induce permanent effects. While malpositions and abnormal pressures are probably of frequent occurrence, it is not likely that any pressure from the uterine wall could produce permanent deformities.

Adams (1873) stated that abnormal conditions in the fetus are induced by powerful emotions in the mother which influence the fetal circulation or produce abnormal fetal secretions.

Broadhurst (1868), and W. T. Little (1881), and others, attempt to account for both congenital and acquired deformities as the result of some type of functional or organic disease of the central nervous system. Lesions due to cerebral diseases or disturbances in the centers of the spinal cord were thought to result in paralyses or spasms in particular sets of muscles of the parts of the body affected, which in turn would cause such maldevelopments as club-feet. This theory was proved untenable by W. Murray ('26) who, through his study of the peripheral motor neurones in a series of mice which exhibited more or less pronounced limb abnormalities, found that the deformities of the appendages were not accompanied by any appreciable deficiency of the motor neurones of the cord at the level of the deformed limb.

Berg ('05) admitted that paralysis due to a disease of the spinal cord is a frequent non-congenital deformity, such as club-feet, but he stated that such a cause is hardly operative in congenital cases, because the leg in congenital talipes does not at all resemble paralysis, a condition in which all of the muscles of the leg are affected and not a single group as is true in cases of congenital club-feet.

The most recent hypothesis concerning the etiology of congenital abnormalities is the one set forth by Streeter ('30), who contends that certain types of abnormalities are caused by amniotic bands in which the fetus becomes entangled, thereby causing parts to become deformed. Streeter also contends that other types of deformities are due to a disparity in the quality or vitality of the different tissues of the body, the tendency to which is inherited through the germ plasm.

In reviewing the literature on this subject, it appears that the reason for so many theories lies in the failure of the investigators to view the problem from the same angle. Had they looked upon the problem from the standpoint of em-

bryonic development and studied the various morphological stages of the developing embryo they would, no doubt, have arrived at different conclusions.

MATERIAL AND METHOD

The nucleus of the stock used for the present investigation was furnished by Dr. C. C. Little, the origin and nature of which has been carefully reported by Little and Bagg ('23, '24), and Bagg ('25, '29), Bagg, MacDowell, and Lord ('25), Bagg and Halter ('27), and from whose accounts the following brief summary is taken.

In the experiments, animals of both sexes were separated into two groups, one group being x-rayed and the other used for controls. Twenty males and ten females were treated. The animals apparently suffered no immediate physiological effects following the treatment, except a period of temporary sterility which lasted about 3 months. The treated animals were mated and produced offspring which exhibited the following types of characteristic deformities:

1. An eye abnormality—which existed in all intergrades between cloudiness or slight reduction in size of the eye to nearly complete atrophy of both eyes.

2. A foot and leg defect—which varied in range from a dwarfing of one digit to complete clubbing and stumping of all four appendages.

3. A 'saddle' abnormality—which consisted in the appearance of areas of short hair on the sides and flanks.

The fact that such abnormalities failed to appear in the stock before x-raying and in the several thousand control animals from the same stock kept under close observations indicated that the x-ray irradiation may be responsible, at least in part, for the abnormalities.

The material used for this study was all carefully timed. With care and experience it was possible to observe insemination so that a sequence of stages of embryos was obtained with some degree of certainty.

The observations of the present investigation are based on a careful examination of the serial sections of 304 embryos and 104 whole specimens, the Bagg albino normal stock being used as controls.

GENERAL STATEMENT

The present observations on the developmental history of this special strain of x-rayed mice began with the pro-nuclear stages. After working over the various stages represented in a large series of embryos ranging in age from the first to the eleventh day, inclusive, and comparing them with similar stages in normal development, it was found that these early stages of development in the x-rayed strain appeared normal in every respect, the abnormalities first appearing after the eleventh day. This being true, a description of these stages seemed unnecessary. However, a careful and detailed study was made of these earlier stages in the hope that some clue might be discovered which would throw light on the abnormalities that occur later in development. No such clue was discovered.

As previously pointed out by Little and Bagg ('23, '24), and Bagg ('25), and confirmed in the present study, the abnormalities always occur on specific parts of the body, the other parts remaining normal throughout development. For this reason a description of only those body parts predestined by heredity to show abnormalities will be given.

PART I

THE FOOT ABNORMALITY

The foot abnormalities first become evident toward the end of the eleventh day. The peripheral capillary net and marginal vein, which in normal material is found embedded deeply in the mesenchyme, has in several instances in the abnormal strain been found to occupy such a superficial position that their endothelial walls were in close proximity to the surface ectoderm (fig. 1). Since the capillary network is normally more extensive in the region of the apex and along

the lateral and mesial margins of the bud, the abnormally located vessels are more abundant in these loci.

The abnormally located peripheral vessels appear normal histologically. The nuclei of their endothelium are distinctly visible and there are no breaks in their walls which might indicate injuries due to their mallocation.

Correlated with the peripheral location of the marginal vein and its branches is a distinct separation of the integument from the underlying mesenchyme. The space formed as a result of the separation is filled in life with an amber-colored fluid which resembles lymph. While the endothelium of the marginal vein actually projects into the space made by the separation, there are no breaks in its wall that would permit a free exudation of the blood from it into the space. The abnormal location of both the peripheral capillaries and the marginal vein are indications of the first stage in the development of the foot abnormalities, but there is no specific evidence that the separation of the ectoderm from the mesenchyme of the limb bud is due to the abnormal location of either the peripheral capillaries or the marginal vein and its branches.

Bleb formation

The next stage in the development of the foot abnormalities is characterized by a further separation of the superficial ectoderm from the underlying mesenchyme. The spaces thus formed become much larger than in the previous stage and are filled with the same amber-colored fluid. The ectoderm has been so lifted that it forms a rather conspicuous protrusion in a blister-like structure or bleb (fig. 2). One might naturally assume that, on account of the close proximity of the peripheral blood vessels to the surface of the mesenchyme, previously referred to, some of them might have ruptured and that the exudation of their contents into the space between the ectoderm and mesenchyme caused a separation of these tissues, thus forming a bleb. In the majority of cases, however, blebs are formed independently of the displaced capillaries.

The blebs may form any time up to the fifteenth or sixteenth day of development, but in the majority of cases they form very soon after the vascular network has been established along the periphery of the limb buds. In no case have they been observed before the eleventh day after insemination.

The blebs vary greatly in size and can be found in intergrades from a slight separation of the two strata to an enormous blister that involves the greater part of the appendage. The only correlation factor that seems applicable is the age of the embryo, for as development progresses the volume of liquid and pressure in the embryonic circulation becomes increased, more blood passes through the peripheral vessels, and as a consequence more diffusion of the plasma may result, tending to increase the size of the bleb. The blebs, likewise, vary in location. They may be found on almost any part of the limb bud, but in the majority of cases they form near the apex or along the lateral or mesial margin of the limb.

The blebs are naturally bounded on their mesial side by mesoderm and on their lateral margin by ectoderm. In the early stages in the formation of the blebs neither the mesenchymal nor the ectodermal cells exhibit any changes due to their presence, but as the blebs grow in size the cuboidal cells of the ectoderm become so drawn out that they are scarcely distinguishable. The mesenchyme bounding the blebs mesially fortifies itself against the fluid of the bleb by an increase in the number of cells in that region.

Hematoma formation

Simultaneously with the increase in the size of the bleb, the collateral branches of the marginal vein, which are but endothelial tubes, grow so close to the surface that they rupture and allow the blood to flow into the bleb, forming a hematoma, or a blood lesion. Thus in a few cases the blebs become blood lesions, but this is not always true. As a result of the entrance of blood into a bleb, the whole structure becomes still larger, noted under operative observation.

Since the blebs and hematomas are formed on the surface and involve chiefly the superficial ectoderm and a small portion of the mesenchyme, the underlying tissues from which the muscle and bone elements develop are not affected. Therefore, it is evident that the blebs and hematomas play only a very minor role in the production of the gross disturbances of the limb, although they do play a small part in the production of other abnormalities which involve primarily the integument.

Thrombi formation

The next phase in the abnormal development of the limbs involves a change quite different from that which causes the formation of the blebs and hematomas. This phase involves a phenomenon similar to thrombosis, which is a peculiar clotting of the flowing blood in the vessels during life. It has been described, so far as I am able to determine, only in man and is not yet clearly understood.

Small agglutinations of corpuscles (fig. 3), which are considered to be the beginnings of the formation of thrombi can be observed in some of the more peripheral veins of the limbs in 13½-day embryos. At this stage the thrombus is primarily a reddish brown mass of corpuscles which seem to be entangled in a sticky homogeneous matrix. The change of coloration is probably due to a breaking down of the hemoglobin of the cells into haematoidin which, according to Virchow (1860), always occurs when blood cells become agglutinated or isolated from other living cells. Through a continuous deposition of corpuscles these agglutinations become larger until finally they completely fill the blood vessel in which they are contained, and as more corpuscles are involved in the coagulum the thrombus grows in size by a distention of the vessel walls. If the blood vessel containing the thrombus is deeply embedded in the mesenchyme the distention is equal in all directions (fig. 4), but in cases where the vessel has a peripheral location, the distention is more extensive toward the surface, resulting in a bulging of the

thrombus from the surface of the limb. It is very common for thrombi to occur in appendages that already contain blebs or hematomas and it is also usual for them to occur close to a bleb or hematoma so that as they develop they project into the already formed blister (fig. 4).

The thrombi, in early stages of formation, do not affect the endothelial wall of the blood vessel involved, but as they grow in size and as distention begins to take place, the endothelial wall and the neighboring mesenchyme begin to degenerate, allowing some of the blood cells of the thrombus to escape through the walls of the vessels and penetrate deeply into the mesenchyme of the limb (fig. 5).

The morphological character of the limb at this age makes the peculiar formation of thrombi possible. It must be recalled that the blood vessels are now but endothelial tubes and the unorganized mesenchymal tissue surrounding them allows free expansion. There are no connective tissue layers or intracellular fibers around the vessels such as develop later, which might inhibit the free distention of the vessels' walls or the extravasation of cells from the enlarging thrombi. In the foci of the limbs where the thrombi become localized and from which blood becomes extravasated, the normal development naturally becomes impaired or perverted. However, this factor is not entirely responsible for the production of the abnormalities, for the mechanical effect of the thrombi themselves, crowding out the forming tissues from their normal position, is undoubtedly a contributing factor involved in the production of the malformations of the limbs. Since the amount of blood extravasation and the extent of its penetration into the tissues is so variable, and since the thrombi may occur in almost any portion of the limb, the variability of the deformities resulting from them is clearly explicable.

About the fifteenth day of development, the lymph supply to the appendage is established, and soon after this the blebs and hematomas begin to show signs of disintegration and become almost completely resorbed before birth.

Thrombi first become injurious after the fifteenth day of development, when the muscular elements of the limb are being laid down. At this time they have completely broken down the vessel walls in which they were contained, allowing a free extravasation of blood into the surrounding tissue. The scleroblastema of the limbs is affected only in rare instances, due to the fact that the thrombi usually form in the superficial blood vessels, and also to the fact that the skeleton of the limb, at least the longer cartilage bones, reaches such an advanced state in its organization before the blood becomes extravasated from the thrombi that it inhibits a free penetration of the blood cells. On the other hand, the muscles or groups of muscles in the limb are just being organized, and these tissues, being less compact, cannot inhibit the enlargement of thrombi or the penetration of extravasated blood between their forming muscle cells. Thus there is an abnormal physiological reaction set up which brings about a retardation or perverted development of the limb muscles.

In embryos after 18 days of development the thrombi for the first time since their formation inhibit a morphological change. The blood cells involved in the agglutination seem very closely compressed and take a light stain. The blood corpuscles exhibit signs of fragmentation and degeneration (fig. 6), a phenomenon known as karyolysis, which ultimately results in the complete liberation of the hematoïdin from the blood cells. This process continues until shortly before birth, when the thrombi appear completely washed out.

Embryos examined 24 to 36 hours before birth show the thrombi in the process of organization, a condition which is essentially the reaction of the limb elements to the thrombus. This process begins by an ingrowth of fibro- and angioblasts from the surrounding connective tissue into the thrombi where an elaborate fibrillar network is formed between the blood cells (fig. 6). Twenty-four hours after birth, the thrombi have completely disappeared, leaving, however, small traces of blood pigment in the connective tissue which enclosed and absorbed them. Canalization represents the last phase in the

organization process and involves a reestablishment of blood vessels in the morbid tissue of the limb (fig. 7).

As shown in figure 7, the skin, which was retarded in its development by the thrombus, is now more or less normal, although it appears somewhat cornified and still contains blood pigments from the thrombus. The connective tissue, which completely fills the space occupied by the thrombus, later established muscular connections, but the formative axis of the limb has become so distorted that it is unable to resume a normal form and the limb thus affected remains deformed throughout the life of the individual.

PART II

THE 'SADDLE' ABNORMALITY

At the beginning of the twelfth day of development, the epidermis in the lumbar region of the back is made up of a double layer of ectodermal cells, a superficial layer, the periderm, and a basal layer, the stratum germinativum, which lies in close contact with but not adherent to the underlying mesenchyme. The underlying mesenchyme is made up of loosely arranged stellate cells, and is supplied with an elaborate vascular network.

The blebs that occur in the region of the back are formed in the same way as those on the limbs. In no case were hematomas found in this region. This is difficult to explain, since the blood supply to the region of the back is very abundant. The blebs, in their development, project out from the body of the embryo and in no way interfere with the underlying strata (fig. 8). They persist but a short time, for no blebs have been observed after the fifteenth day of development, which indicates that they are absorbed even sooner in the region of the back than in the limb region. This is explicable on the basis that the lymphatic system is established much earlier in development in the body region than in the limbs.

Groups of agglutinated blood corpuscles are present in some of the dermal blood vessels. These appear to be adher-

ing to the vessel wall and are recognizable as the beginning of the formation of thrombi. The thrombi in the region of the back always form in the dermal layer of the skin where they remain throughout their existence.

Through a continuous hypostasis of the blood corpuscles, a thrombus gradually grows in size until finally the blood vessel in which it is formed becomes completely occluded. The continuous pressure on the column of blood causes more corpuscles to become agglutinated and the thrombus to increase so much in size that its investment is forced to expand (fig. 9). The fibrous connective tissue of the corium at this time is so loosely held together as to allow the thrombus free expansion. The distention, however, is mainly toward the periphery and the underlying structural elements of the embryo are usually not seriously affected by their formation.

Soon after the eighteenth day, the thrombi have reached the full extent of their development. The connective tissue forming the investment around them has become so fibrous as to inhibit further distention. It is at this time that the hair germs normally begin their development. They appear as localized epithelial thickenings or ectodermal buds which soon after their formation grow downward into the underlying dermis where the hair follicles will later develop.

While the thrombi form in the blood vessels of the dermis, they become so enlarged in the course of their development that the mesenchyme of the dermis, bounding them externally becomes greatly reduced leaving only a thin layer between the thrombus and the superficial ectoderm (fig. 9). Where thrombi are present the hair germs begin their development normally, but, due to the fact that their subsequent growth downward is inhibited by the proximity of the thrombus (fig. 10), they remain in this early developmental stage until the thrombi are absorbed.

Organization of the thrombi takes place in the same manner as described for the thrombi of the limbs. After the thrombi begin to organize, the hair germs, whose development was earlier stopped by the thrombi, continue their development

and as the mesenchyme of the dermis is reestablished under the skin they gradually grow downward and form papillae and follicles in a more or less normal manner.

About the third day after birth, the hair appears on the surface as a very fine lanugo. The real effect of the thrombi can be seen at this time, for in those regions of the body where they occurred there is a distinct retardation in the appearance of hair through the skin, although the skin itself appears perfectly normal.

Since the hairless areas often are present on both sides of the back with a strip of hair extending down the mid-dorsal line, the saddle-like appearance results which Little and Bagg ('23, '24) called the 'saddle' abnormality.

The saddle abnormality persists only for a short time, after the fifth day the lanugo in the short-haired areas begins to grow and at the end of the second week has reached a length equal to that of adjacent parts of the body.

PART III

THE EYE ABNORMALITY

Thrombi appear at various loci on the head about the time they appear in other regions of the body (15 to 17 days) and go through the same course of development. The underlying structures of the head are, as a rule, not interfered with, but in cases where thrombi form in regions of the eye distinct detrimental effects result. Since the lens of the eye normally forms before the fifteenth day of development and soon migrates to a position in the optic cup, the thrombi which appear later can in no way affect its formation. It is not until the eighteenth day that the thrombi really affect the development of the eye to any extent. The eyelids at 18 days begin their formation as indentations above and below the eyeball, and when figure 11, which shows a thrombus formed over the developing eye, is examined, it becomes evident that the thrombus has completely inhibited the formation of the eyelids. Thrombi, however, are not always so located that they inhibit the formation of both eyelids, but may interfere

with only one lid, the other one developing normally. In the majority of cases the thrombus in the region of the eye enlarges by becoming further distended from the head, but sometimes the thrombi seem to exert a marked pressure on the eye pressing it closely against the fore-brain (fig. 12). Under such conditions, not only the eye, but also the side of the head is affected.

A thrombus in the region of the eye is absorbed more slowly than a thrombus located elsewhere on the body. This is probably due to the fact that both the lymph and blood supply to the eye are very limited. Their organization begins soon after the eighteenth day, by the breaking down of the nuclei of the blood cells involved. In other regions of the body, this process was followed by a proliferation of the surrounding connective tissue into the thrombus, forming a fibrillar network. This, however, is not the case with thrombi in the eye region. This deviation from the regular process of organization is probably due to the small amount of connective tissue present in the dermis in the head region, resulting from the promixity of the brain to the surface. The organization process, however, continues with hemolysis of the blood cells until at 24 hours before birth the thrombi have become greatly reduced in size (fig. 13). As the organization process continues and the thrombus becomes absorbed, the eye itself suffers greatly from the mechanical effects produced. The mesenchymal tissue between the lens and the thrombus becomes insufficient to resist the constant pressure exerted by the latter and therefore breaks down, allowing a free exudation of blood cells from the thrombus around the lens and into the inner chamber of the eye. This results in a violent reaction of the parts of the eye in contact with the extravasated blood, which, as shown in figure 13, results in a marked distortion of the lens and the inner retinal coat of the eye.

At birth, the corpuscles have been hemolyzed and the thrombus has been transformed from a cellular state to that of a viscous fluid. There is also a marked ingrowth of a fibrillar substance into the remains of the thrombus. The

thrombus of the eye region thus becomes transformed into a fibrous network before it is finally absorbed soon after birth.

The eye thus affected in its development by the presence of a thrombus, atrophies soon after birth and the orbit becomes filled with connective tissue.

All the eye abnormalities are not as extensive as the one described in the preceding paragraphs, for in some cases a thrombus affects only a small portion of the eye, and as a result of the early absorption of the thrombus, the eye suffers little or no ill effects. In the majority of cases, however, the eye defects result in blindness.

PART IV

ETIOLOGY OF THE ABNORMALITIES

The histogenesis of the limb, skin, and eye abnormalities has been discussed in the foregoing sections and the histological changes have been followed from their embryonic origin to a period shortly after birth. It is the purpose of this section to give, as far as possible, the etiology of the abnormalities in question.

Bagg ('29) and Bonnevie ('30) have stated that the abnormalities occurring in the experimental strain of mice are due entirely to bleb formation, but the present investigation has shown definitely that thrombi and not the blebs are the responsible factors. Bagg and Bonnevie failed to differentiate between blebs and thrombi; they called all the pathological structures blebs. They stated too, that the blebs are formed between the ectoderm and mesenchyme, yet Bonnevie ('30) in plate II, figure D, 4b., illustrates a thrombus formed over a developing eye, which is bounded completely by mesoderm. She failed to recognize this fact and called the pathological structure a bleb. No doubt both Bagg and Bonnevie would have arrived at different conclusions had they made a careful histological study of the early stages of development, where the blebs and thrombi are readily distinguishable as structures having a distinctly separate origin.

Since thrombi always develop in the same definite way, irrespective of their location, one is led to believe that the agent which is responsible for their formation is general throughout the body of the embryo.

The phenomenon of thrombosis is well known to the pathologist, but it must be admitted that little is known concerning its real cause. It is the accepted belief that thrombi are usually formed as a result of an injury to the endothelium of the vessel walls. Thrombocytes are subsequently deposited in these loci of injury and they in turn bring about an agglutination of the blood corpuscles which come in contact with them, thus resulting in a coagulation of blood which increases until the vessel eventually becomes completely occluded.

It would be of particular interest to know why the thrombi form in this special strain of deformed mice. Since the frequency of abnormalities is greater in the progeny of abnormal parents than in those of the normal members of the strain, there must be some factor in the parents that varies in degree of intensity with the degree of the abnormalities.

It will be recalled that the blebs, which might or might not later become hematomas, always form at about the same stage in development, and that thrombi appear very soon thereafter. Although these structures arise independently and are correlated in no way morphologically, it seems possible that the factors influencing one to form might also cause the formation of the other a short time later. For this reason, and since the blood is involved in the formation of both hematomas and thrombi, blood counts were made in both the x-rayed strain and the normal strain in embryos at various stages during gestation, and in adults, in an attempt to discover some vascular defect which might be correlated with the occurrence of these abnormalities.

Those individuals showing the greatest number of abnormalities were considered most desirable. Both red and white corpuscle counts were made in each mouse, and counts were made in fifteen individuals in each group.

Result of blood counts

The results of the comparative red and white blood corpuscle count in the x-rayed strain and normal albino embryos at various stages of gestation are given in tables 1 and 2.

Embryos 11 days old. It has been shown in the text that embryos of the x-rayed strain develop normally up to the twelfth day after insemination. In view of this fact, embryos 11 days old were selected as a starting point for the blood counts. 1) Comparison of the red counts: The data given in table 1 show a difference in the means of the two strains of $-70,000 \pm 72,000$ corpuscles per cubic millimeter of blood. The difference between the two means is 0.97 times its P.E., which indicates that there is no significant difference between the two means. 2) Comparison of the white counts: The data recorded in table 2 show a difference in the means of -54 ± 196 corpuscles per cubic millimeter of blood. The difference between these two means is 0.27 times its P.E., indicating that there is also no statistically significant difference between these two means.

Embryos 13½ days old. It has been shown that blebs form on some of the embryos of the x-rayed strain soon after the twelfth day of development. Since some members of a litter exhibit blebs and some do not, these groups will be taken up separately and their blood counts compared with the normal.

Embryos of the x-rayed stock not having blebs compared with the normal albino: 1) Comparison of red counts: The data obtained in these counts as recorded in table 1, show a difference of the means of $40,000 \pm 93,000$ corpuscles per cubic millimeter of blood. The difference between the two means is 0.43 times its P.E., which indicates no significant difference. 2) Comparison of the white counts: The data obtained in these counts reveal a difference of the mean of -27 ± 240 corpuscles per cubic millimeter of blood. The difference between the two means is 0.11 times its P.E., indicating no significant difference between the two means.

Embryos of the x-rayed stock having blebs compared with the normal: 1) Comparison of red counts: The data obtained in these counts show a difference of the means of $2,429,000 \pm 66,000$ corpuscles per cubic millimeter of blood. The difference between the two means is 36.80 times its P.E., which indicates a significant difference between the two means. 2) Comparison of white counts: The data obtained reveal

TABLE 1
Comparative red blood counts of the x-rayed and normal strains

	LOW	HIGH	MEAN AND P.E.	DIFFERENCE OF MEANS
Embryos 11 days old:				
Normal	1500 ¹	2500	2050 ± 50	
X-rayed	1500	2550	1980 ± 52	-70 ± 72
Embryos 13½ days:				
Normal	1450	2400	2043 ± 52	
(No blebs) x-rayed	1250	2700	2083 ± 77	40 ± 93
(With blebs) x-rayed	3950	4750	4472 ± 40	2429 ± 66
Embryos 17 days old:				
Normal	2450	4200	3285 ± 99	
(With blebs) x-rayed	3700	4900	4376 ± 67	1091 ± 120
Embryos 21 days old:				
Normal	3450	4800	4355 ± 74	
(With blebs) x-rayed	3355	4825	4338 ± 78	-17 ± 108
Embryos at birth:				
Normal	4080	6440	5302 ± 127	
(Abnormal) x-rayed	4930	6800	5481 ± 85	179 ± 153
Mice at 8 months:				
Normal	5520	13980	9828 ± 422	
(Abnormal) x-rayed	6960	13150	10960 ± 315	1132 ± 527

¹ The last three zeroes have been omitted from each of the numbers in this and the next table.

a difference of the two means of 1574 ± 215 corpuscles per cubic millimeter of blood. This difference is 7.32 times its P.E., and is significant.

Since the embryos of the x-rayed stock which do not exhibit blebs are normal so far as their blood counts are concerned, they were not included in the comparative blood study of the later stages of embryos.

Embryos 17 days old. 1) Comparison of the red counts: The data obtained in making the counts show a difference of the two means of $1,091,000 \pm 120,000$ corpuscles per cubic millimeter of blood. The difference of the two means is 9.09 times its P.E., which indicates that the difference is significant. 2) Comparison of the white counts: The data obtained in making these counts show a difference of the two means

TABLE 2
Comparative white blood counts of the x-rayed and normal strains

	LOW	HIGH	MEAN AND P.E.	DIFFERENCE OF MEANS
Embryos 11 days old:				
Normal	740	3300	2040 ± 139	
X-rayed	800	3340	1986 ± 138	-54 ± 196
Embryos $13\frac{1}{2}$ days:				
Normal	750	3900	2400 ± 170	
(No blebs) x-rayed	900	3940	2373 ± 170	-27 ± 240
(With blebs) x-rayed	2400	5140	3974 ± 131	1574 ± 215
Embryos 17 days old:				
Normal	2100	5240	3666 ± 171	
X-rayed	2300	5340	3837 ± 156	177 ± 231
Embryos 21 days old:				
Normal	1975	6675	4159 ± 233	
X-rayed	2000	6275	3802 ± 255	-357 ± 345
Embryos at birth:				
Normal	2800	9980	5205 ± 309	
(Abnormal) x-rayed	3060	9160	6325 ± 283	1120 ± 419
Mice at 8 months:				
Normal	6000	11000	7980 ± 276	
(Abnormal) x-rayed	4600	10920	7670 ± 303	-310 ± 410

of 177 ± 231 corpuscles per cubic millimeter of blood. The difference of the two means is 0.76 times its P.E. and is not significant.

Embryos 21 days old (just before birth). 1) Comparison of the red counts: The data obtained in making the counts reveal a difference of the two means of $-17,000 \pm 108,000$ corpuscles per cubic millimeter. The difference between the

two means is 0.16 times its P.E., which indicates that the difference in the two means is not significant. 2) Comparison of the white cell count: The data obtained show a difference of the two means of -357 ± 345 corpuscles per cubic millimeter of blood. The difference of the two means is 1.03 times its P.E. and is not significant.

Embryos just after birth, and mature mice. The data as recorded in tables 1 and 2 obtained from blood counts of the abnormal members of the x-rayed stock, both at birth and at maturity, when compared with data of normal albino mice at the same respective ages, reveal that there is no significant difference between the two strains.

Discussion and summary of the results

The results obtained in making the comparative blood counts between embryos of the x-rayed and normal Bagg albino strains, reveal certain definite results. The first is that the embryos of the x-rayed stock at 11 days of development are normal as far as their blood counts are concerned, as well as in appearance.

When the results from a study of the blood counts of embryos $13\frac{1}{2}$ days old are compared, we find that the means obtained from normal members of the x-rayed stock coincide very closely with those obtained from embryos of the Bagg albino stock. This indicates that the normal members of the x-rayed stock are normal so far as their blood counts are concerned. But when the mean counts recorded from the bleb exhibiting members of the x-rayed stock are compared with the mean counts obtained from embryos of the Bagg albino stock, a very significant difference in the blood of these two strains of mice at this stage of development is indicated.

From the onset of the present investigation we have been confronted with the problem of definitely determining the source of the liquid which accumulates under the superficial ectoderm to form the blebs. The increase in the number of corpuscles per cubic millimeter in the blood of those embryos of the x-rayed stock which exhibit marked blebs is very

unique, since the blood counts of those members of the x-rayed stock which do not show blebs remain practically normal. The blood count is higher in those individuals possessing larger and more numerous blebs. It becomes evident that the liquid part of the blood escapes from the circulation and accumulates in these blister-like structures, this loss of fluid from the blood raising the cell count, the extent depending of course upon the amount of fluid extravasated. It is to be recalled that the embryos of each litter chosen for the count were selected entirely in accordance with the degree of bleb formation which they exhibited.

Bonnevie ('30), working on a stock of x-rayed mice, also obtained from Dr. C. C. Little, formulated a hypothesis that the fluid accumulated in the blebs has its origin in the cerebro-spinal tube. She states that in her strain of mice some of the embryos accumulate an excess of fluid in the medullary tube and that in later stages when the brain lumen becomes decreased the excess fluid is pressed out through the foramen anterius and accumulates under the epidermis to form a large bleb on the head. She further states that the bleb thus formed spreads to other regions of the body forming localized blebs. If an excess fluid does accumulate in the brain cavities of embryos of this experimental strain, as pointed out by Bonnevie, such cases are evidently the exception and not the rule, for no such condition has been found in my material. It might be stated here that many cases have been observed where blebs are present on the feet and regions of the body with no sign of one existing or having been present on the head. Such cases cannot be explained by the hypothesis set forth by Bonnevie.

The fact that there is such an increase in the viscosity of the blood along with the appearance of the blebs on the body shows clearly that the amber-colored fluid which fills the blebs undoubtedly has its origin from the blood stream and may safely be called extravasated plasma.

The results thus far obtained and analyzed reveal the source of the liquid which fills the blebs. The problem still

remains as to what factor or factors are present in the embryos of the x-rayed stock about 15 days after insemination which causes the thrombi to form. It has been pointed out that thrombi in no case have been found to occur in individuals that did not also show blebs. This fact leads one to believe that there must be some correlation between the presence of blebs and the formation of thrombi. The fact that blebs are, as a rule, formed from 2 to 3 days before the thrombi appear leads one to assume that the blebs are predisposing factors to the formation of thrombi. As already pointed out, the blebs are formed apparently as a result of an escape of the plasma from the blood stream and its accumulation in the various loci of the body. As a result of the extravasation of plasma from the circulation the viscosity of the blood is proportionally increased, bringing about a polycythemic condition. In cases of polycythemia, the viscosity of the blood is so markedly increased that it flows very slowly through the smaller vessels, the more peripheral vessels of the circulation in some cases being completely stopped. This sluggish circulation, and in some cases complete stopping of the blood flow, results in the stagnation of the blood in the smaller peripheral vessels and consequently a lowered oxidization in the surrounding tissues, a condition which eventually leads to injury of the endothelium of the blood vessels.

It has been shown by Eberth and Schimmelbusch (1888), Welch ('00), Aschoff ('12), and MacCallum ('28), and others, that thrombi are formed mainly as a result of an injury to the endothelium of the blood vessels leading subsequently to an accumulation of thrombocytes which brings about an agglutination of corpuscles.

The evidence previously pointed out proves that the thrombi are the immediate factors in the production of the abnormalities of the limbs, skin and eyes, and that polycythemia is the causative agent in the formation of the thrombi. The comparative blood study shows that the polycythemia is brought about by an escape of the plasma from the blood to form

the blebs, but a causative factor for such an excessive extravasation of the plasma has not been found.

The capillary walls are normally permeable to plasma, allowing it to flow into the inter-cellular spaces in the form of lymph. However, the condition here described seems to be that of a more than normal permeability, so that the fluid part of the blood is allowed to drain out to the extent that it becomes so viscous as to be injurious to the embryo. The smaller arterioles and capillaries have been examined in great detail and since no morphological anomaly has been discovered, it is possible that some physiological factor is involved.

It has been definitely proved through many years of breeding experiments by Little, Bagg, Murray, and others, as well as in connection with the present work, that the feet, eye, and skin abnormalities are heritable. One may postulate that they originally appeared as a result of a mutation of such a nature as to bring about a hypoplasia of the vessel walls. It is hoped that subsequent investigations may enable us to determine definitely the cause of the hypoplasia, if it be purely morphological or due possibly to some heritable endocrine disturbance which results in an abnormal physiological condition in the vessel walls.

Streeter ('30) in discussing intra-uterine deformities states that no evidence has been found to indicate that all congenital deformities are due to amniotic bands, adhesions, or other mechanical conditions. He states that there is an extreme disparity in the quality or vitality of the different tissues of the body, which is inherent in the germ-plasm and transmitted to the succeeding generations, and occasionally one encounters cases where a tissue is given an excess vitality or growth vigor, which results sometimes in that tissue becoming twice its normal size. The opposite also may happen, where an extreme deficiency results to the extent that a given tissue or organ simply does not develop (congenital atrophy or amputation).

If the hypothesis set forth by Streeter is correct, then it can be said that there may be no correlation between the etiology of congenital abnormalities as they appear in man and as they appear in this experimental strain of x-rayed mice. But Streeter has formulated these hypotheses from an examination of fetuses in which the abnormalities have already occurred. While it may be tenable that the congenital abnormalities as they appear in man are due entirely to the disparity of the vitality or quality of the tissues of the embryo, Doctor Streeter has not presented conclusive evidence to substantiate this hypothesis. It is, however, possible that some factor, pathological or otherwise, present in the human embryo may exert an influence on the very early formation of those organs which later become abnormal. Such a factor could, of course, be discovered only through a careful study of earlier stages of development. They might be found to be of the same nature as the blebs and thrombi found in this x-rayed strain of mice.

SUMMARY

Morphological

I. An extensive histological study was made of the embryonic stages of a strain of abnormal x-rayed mice, extending from the pro-nuclear stage to several hours after birth. The deformities in question occur as congenital abnormalities involving principally the feet, skin and eyes.

II. Development of the abnormal strain is apparently normal until after the eleventh day following insemination.

III. In embryos about 11 days old peripheral capillaries and the marginal vein of the limb buds are often found located in an abnormal position close against the superficial ectoderm.

IV. In embryos after the beginning of the thirteenth day of development, the following characteristic pathological structures appear:

1. Blebs are formed as a result of an accumulation of plasma-like fluid between the superficial ectoderm and the underlying mesenchyme.

2. In some cases, adjacent blood vessels rupture and the blood exudes into the bleb to form a blood lesion or hematoma.

3. Blebs and hematomas may occur at almost any locus on the body and are variable in size.

4. Small agglutinations of corpuscles become discernible in some of the peripheral vessels, and through a continuous hypostasis the aggregate of corpuscles becomes larger forming a thrombus.

V. Blebs are absorbed soon after the establishment of the lymphatic system.

VI. Blood cells of the hematoma are hemolyzed soon after the eighteenth day of development and are readily absorbed.

VII. Blebs and hematomas are in no way concerned with the production of gross abnormalities of the limb, and play a very minor role in bringing about the skin and eye abnormalities.

VIII. Thrombi are the immediate factors causing deformities in the various regions of the body.

1. Thrombi exert a mechanical effect by crowding out the normal tissues and a chemical effect by the extravasation of blood cells and fluids into the forming tissues, resulting in perverted development.

2. The extent of the deformities produced is dependent upon the size and location of the thrombus involved, and the extent of the extravasation of blood from them.

IX. Organization of the thrombi proceeds in the following manner:

1. A karyolysis of blood cells, resulting in the liberation of blood pigment.

2. An ingrowth of angio- and fibro-blasts which penetrate the thrombus and form a fibrillar network. This process is accompanied by hemolysis or fragmentation of the blood cells.

3. Canalization of the newly-formed fibrillar network.

Experimental

I. Comparison of blood counts of the x-rayed and normal (Bagg albino) strains of mice.

1. Blood counts of individuals of the x-rayed strain 24 hours after birth and at 8 months of age do not differ significantly from the normal.

2. Embryos of the x-rayed strain at 11 days do not differ significantly from the normal.

3. Embryos of the x-rayed strain which do not show blebs are not only normal in appearance but they are also normal as far as their blood counts are concerned.

4. Embryos of the x-rayed strain, which show blebs, show a marked increase in their red and white blood cell counts.

5. Abnormal embryos of the x-rayed strain at 17 days show a blood count higher than the normal.

6. The blood counts of abnormal embryos of the x-rayed strain at 21 days do not differ significantly from the normal.

Conclusions

I. The morphological study has shown that the thrombus is the immediate factor in bringing about the gross abnormalities of the limbs, skin, and eyes.

II. The correlation established between bleb formation and the polycythemic condition of the blood at $13\frac{1}{2}$ days indicates that the fluid which accumulates in the blebs is extravasated plasma.

III. The abnormal permeability is probably due to a hypoplastic condition of the blood vessel walls.

IV. It is not definitely proved whether the hypoplasia of the blood vessels is due entirely to a mutation governing the development of blood vessels directly or to some endocrine disturbance of a hereditary nature.

LITERATURE CITED

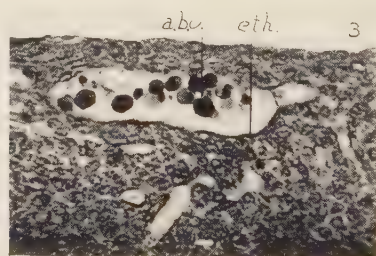
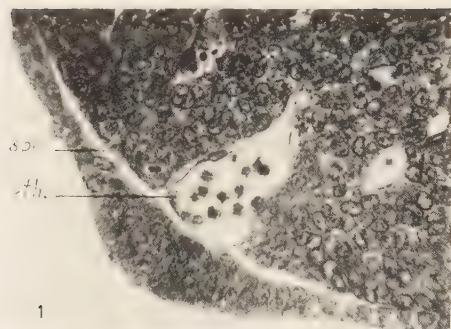
- ADAMS, WILLIAM 1873 Club-foot: Its cause, pathology, and treatment. London.
- ASCHOFF, L., B. VON BECK, O. DE LA CAMP, AND B. KRÖNIG 1912 Beiträge zur Thrombosefrage. Leipzig.
- 1925 Hereditary abnormalities of the viscera. I. A morphological study with special reference to abnormalities of the kidneys in the descendants of x-rayed mice. *Am. J. Anat.*, vol. 36, pp. 275-312.
- 1929 Hereditary abnormalities of the limbs, their origin and transmission. *Am. J. Anat.*, vol. 43, pp. 167-220.
- BAGG, H. J., AND C. R. HALTER 1927 Further studies on the inheritance of structural defects in the descendants of mice exposed to Röntgen-ray irradiation. *Anat. Rec.*, vol. 37, p. 183.
- BAGG, H. J., AND C. C. LITTLE 1924 Hereditary structural defects in the descendants of mice exposed to Röntgen-ray irradiation. *Am. J. Anat.*, vol. 33, pp. 119-145.
- BAGG, H. J., E. C. MACDOWELL, AND E. M. LORD 1925 Further experiments with x-rayed mice and their descendants. *Anat. Rec.*, vol. 31, p. 342.
- BERG, HERMANN 1905 Ueber Muskelatrophie bei Thomsenscher Krankheit. Bonn, 1905.
- BONNEVIE, KRISTINE 1930 Vererbbares Cerebrospinaldefekt (?) Bei Mäusen, mit Sekundären Augen- und Fuss-Anomalien, Nebst Turmschädelanlage. - *Avh. Norske Videnskaps-Akademi I Oslo, I. Mat.-Naturv. Klasse* 1930 No. 13.
- BROADHURST, B. E. 1868 Bemerkungen über die Ursachen der in frühester Kindheit eingetretenen angeborenen Deformitäten. *J. F. Kinderkr.*, Erlang, 1868, S. 353-360.
- EBERTH, C. J., AND C. SCHIMMELBUSCH 1888 Die Thrombose nach Versuchen und Leichenbefunden. Stuttgart.
- LITTLE, C. C., AND H. J. BAGG 1923 The occurrence of two heritable types of abnormality among the descendants of x-rayed mice. *Am. J. Röntgen. and Rad. Ther.*, vol. 10, pp. 975-989.
- 1924 The occurrence of four inheritable morphological variations in mice and their possible relation to treatment with x-rays. *J. Exp. Zool.*, vol. 41, p. 45.
- LITTLE, W. J. 1881 On the etiology of congenital club-foot. *Tr. Internat. M. Cong.*, 7 sess., London., vol. 2, pp. 412-416.
- MACCALLUM, W. G. 1928 A textbook of pathology. Philadelphia.
- MARTIN, F. 1839 Memoirs sur l'étiologie du pied-bot. Paris.
- MURRAY, W. S. 1928 Studies of developmental anomalies in the descendants of x-rayed mice. *Papers of Mich. Acad. of Sci., Arts and Letters*, vol. 10, pp. 509-587.
- STREETER, G. L. 1930 Focal deficiencies in fetal tissues and their relation to intra-uterine amputations. *Carnegie Inst. of Wash., Contr. to Embryol.*, vol. 22, nos. 126-133, pp. 1-44.
- VELPEAU, A. A. L. 1847 New elements of operative surgery. New York.
- VIRCHOW, R. L. 1860 Cellular pathology. London.
- WELCH, W. H. 1900 Thrombosis in a system of medicine. Edited by Thomas Clifford Alburt, vol. 6, pp. 155-228.

PLATES

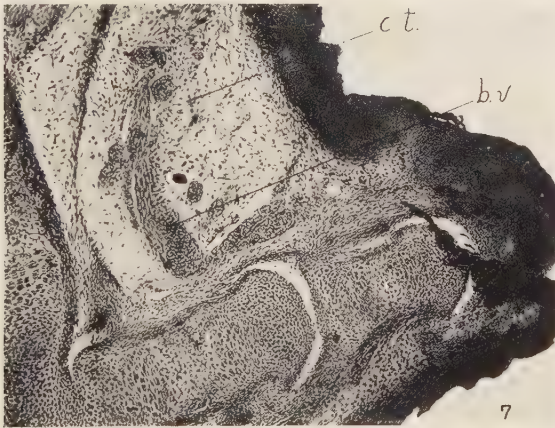
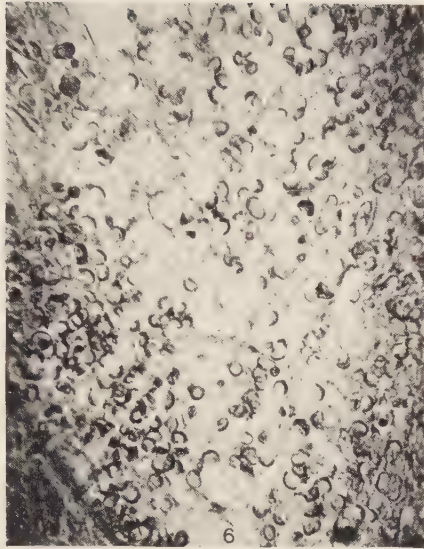
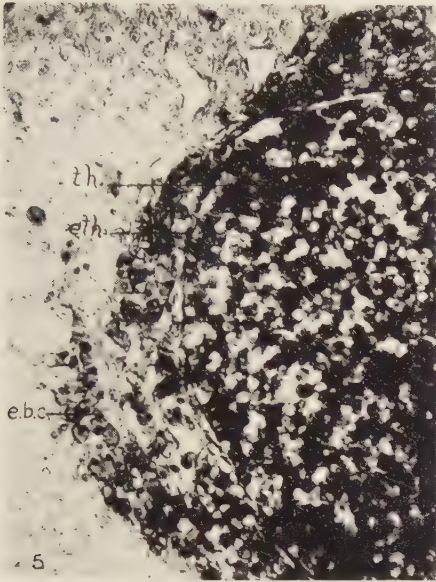
ABBREVIATIONS FOR ALL FIGURES

a.b.c., agglutinated blood cells
b.d., base of dermal layer
bn., bone
bl., bleb
b.v., blood vessel
c.t., connective tissue
d., dermis
e., eye
e.b.c., extravasated blood cells

ect., ectoderm
el., eyelids
eth., endothelium
h.g., hair germ
i.r., inner retinal layer
m., mesoderm
s.ct., subcutaneous layer of the skin
spc., space
th., thrombus



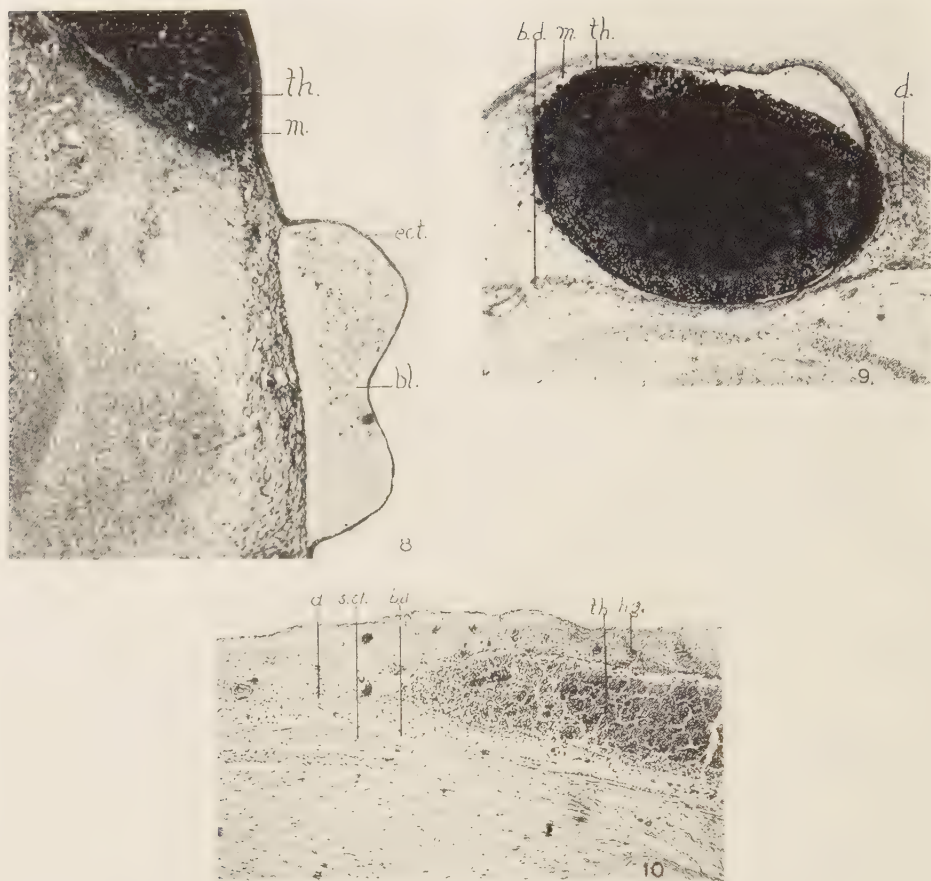
- 1 Photomicrograph of distal portion of a limb bud showing the terminal blood vessel displaced against the superficial ectoderm. Embryo no. 139A, 10 days, 20 hours old.
- 2 Photomicrograph of a section of a limb showing two blebs, one just after its formation, the other more advanced in development. Embryo no. 160, 13 days, 12 hours old.
- 3 Photomicrograph of a section of a limb showing blood corpuscles becoming agglutinated in a blood vessel to form a thrombus. Embryo no. 163, 13 days, 5 hours old.
- 4 Photomicrograph of a section of a limb showing two thrombi, one projecting into a bleb. Embryo no. 227B, 14 days old.



5 Photomicrograph of a section of a limb showing blood cells extravasated from a thrombus into the surrounding tissue.

6 Photomicrograph of a section of a limb showing a thrombus being organized. Embryo no. 219, 19 days, 5 hours old.

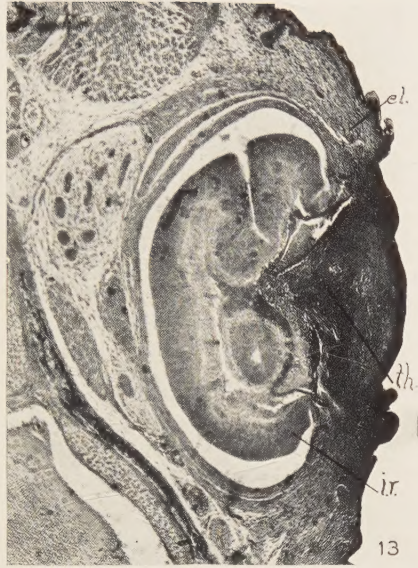
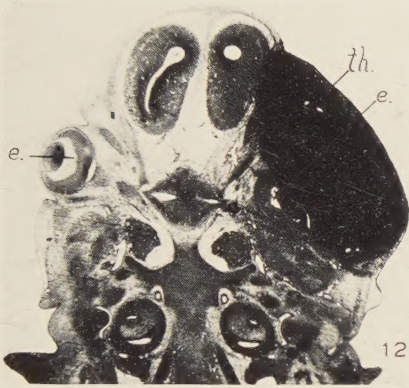
7 Photomicrograph of a section of a limb showing canalization of the connective tissue which replaced an earlier thrombus. Young no. 228B, 24 hours old post partum.



8 Photomicrograph of a section of the skin showing a bleb protruding from the body, and also a thrombus which has extended inward. Embryo no. 227, 13½ days old.

9 Photomicrograph of a transverse section through the body showing a thrombus in the dermal layer of the skin. Embryo no. 216, 17 days, 8 hours old.

10 Photomicrograph of a section through the skin showing a thrombus in the dermal layer inhibiting the downward growth of the hair germ. Embryo no. 222, 18 days, 5 hours old.



11 Photomicrograph of a cross section through the eye showing a thrombus inhibiting the formation of the eyelids. The thrombus here shown has been punctured, allowing some of the blood to escape. Embryo no. 213, 15 days old.

12 Photomicrograph of a section through the head showing a large thrombus forming over one eye. Embryo no. 80, 16 days old.

13 Photomicrograph of a section through the eye showing a thrombus being organized. The pressure of the thrombus has resulted in the distortion of the inner layer of the retina. Embryo no. 215, 20 days old.

